Published in Journal of Agricultural & Food Chemistry, Mar–Apr 1984, Pages 274–276, by the American Chemical Society

Varietal Differences in Distribution of Quercetin and Kaempferol in Onion (*Allium cepa* L.) Tissue

Alexander Bilyk,* Paula L. Cooper, and Gerald M. Sapers

The quercetin and kaempferol contents of eight onion varieties (*Allium cepa* L.) were determined. The dry skins, outer rings, and inner rings were separated and extracted with methanol to obtain flavonol glycosides that were then hydrolyzed to aglycons. Flavonols were detected and quantified by thin-layer chromatography, high-performance liquid chromatography, and spectrophotometric analyses. The quercetin content of onion tissue decreased from the dry skin to inner rings. The skin of all varieties contained quercetin, both as the aglycon and as glycosides; with some varieties, the skin also contained small amounts of kaempferol. Outer rings of all varieties except Red Hamburger contained small amounts of quercetin while those of Early Yellow Globe, Sweet Spanish Hybrid, and Walla Walla contained small amounts of kaempferol as well. Traces of these flavonols also were detected in the inner rings. The highest quercetin content found in the edible portion of these samples was about 60 mg/kg fresh weight. Small quantities of quercetin and kaempferol were present in scallion leaves but not in bulb tissue. Myricetin was not detected in any sample.

In recent years some concern has been expressed regarding certain flavonols that were reported to be mutagenic by the Ames test (Hardigree and Epler, 1978; MacGregor and Jurd, 1978). Among these compounds, which are widely distributed within the plant kingdom (Harborne et al., 1975), quercetin, kaempferol, and myricetin are commonly found in vegetables and fruits (Herrmann, 1976). These flavonols differ only in the number and position of hydroxyl groups attached to the B ring. In plant cells the flavonols occur as glycosides, with sugars bound usually at the C_3 position. The formation of flavonol glycoside normally depends on the action of light (Siegelman, 1964; Mohr, 1969), so that in general the highest concentrations of these compounds occur in leaves while only traces are found in parts of the plants below the soil surface.

The objective of the present study was to determine the distribution of quercetin, kaempferol, and myricetin in different tissues of representative onion cultivars.

EXPERIMENTAL SECTION

Sample Preparation. Seven cultivars of common onions and one scallion type were investigated. Each onion sample was divided into three portions: the dry skin, the outermost rings (scales 1–3), and the remaining rings. In the scallion type onion only leaves and bulbs were investigated. Each portion was diced and extracted by steeping in absolute methanol at room temperature for about 24 h by using a 1:40 ratio (fresh weight:volume) for onion skin and a 1:5 ratio for the edible portions.

Chlorophyll and waxy materials were removed from the methanolic extracts by adding activated carbon (Norit 211 F.Q.P.; Eastman Kodak Co.), in the proportion of 1–2% based on the fresh weight of the onion sample, and filtering through a medium-porosity fritted glass filter funnel under suction. We previously determined that there was no loss of flavonols due to the carbon treatment of onion extracts by spiking extracts with known amounts of quercetin and rutin and measuring recovery (>98%).

Since quercetin is known to occur in the free form in onion skin (Herrman, 1976), its concentration in the crude extract was measured directly by high-performance liquid chromatography (HPLC). Solvent was removed from the crude extract by vacuum distillation at 35 °C. Flavonol glycosides in the residue were hydrolyzed with 2 N HCl by the procedure of Koeppen and Herrmann (1977), and the resulting aglycons were extracted 3 times with ethyl acetate which was also removed by vacuum distillation. The flavonols were isolated by thin-layer chromatography (TLC). The TLC separation was performed on 500-μm thick silica gel coated plates (Analtech, Inc., Newark, DE). The hydrolyzed sample (100 mg) was dissolved in methanol and applied to the plate by means of a streaker (Applied Science Laboratories, Inc., State College, PA). Various TLC solvent systems (mixtures of benzene, pyridine, methanol, ethanol, 2-propanol, formic acid, acetic acid, and water) were compared for the separation of quercetin, kaempferol, and myricetin. Benzene–pyridine–formic acid (65:25:10), the most effective mixture, was selected as the developing solvent. The individual TLC bands, located visually by their yellow or brownish color, were scraped from the plate, extracted with methanol, and examined by HPLC and with a Perkin-Elmer Model 552 recording UV–visible spectrophotometer between 200 and 650 nm. The absorbance of sample solutions was measured against an absolute methanol blank. Identification was based on the comparison of chromatographic and spectral data for unknowns and standards (Sigma Chemical Co., St. Louis, MO). Quantitative analyses for flavonols were performed by HPLC, using an external standard quantitation procedure. A Waters HPLC system (Waters Associates, Milford, MA) consisting of two Model 600A pumps, a Model 660 solvent programmer, a Model U6K injector, a Model 440 absorbance detector set at 280 nm, and a 3.9 mm i.d. × 30 cm chromatographic column packed with μBondapak C_{18} (particle size, 10 μm), in combination with a Hewlett-Packard 3390A integrator, was used. A guard column packed with Bondapak C_{18} Corasil (Waters) was installed in front of the analytical column. The eluant was water–acetic acid–methanol (42:8:50) at a flow rate of 1 mL/min (Wulf and Nagel, 1976). Based on the quantitative data obtained by HPLC, the contents of detected flavonols (g per kg of onions, fresh weight) were calculated for each portion of onion and for the combined edible portions.

RESULTS AND DISCUSSION

The R_f values of flavonols in onion extracts, separated by TLC, were compared with R_f values of the three

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

Table I. Onion Flavonols (g/kg of Onion Portion, Fresh Weight)[a]

variety	portion	quercetin		total kaempferol
		in free form	total	
Carmen Hybrid	A	7.73 (23%)	34.15 ± 0.460	0.677 ± 0.040
	B		0.062 ± 0.006	ND
	C		0.027 ± 0.001	ND
Sweet Spanish Utah	A	3.87 (24%)	16.53 ± 0.410	0.123 ± 0.002
	B		0.295 ± 0.005	ND
	C		0.002 ± 0.001	ND
Early Yellow Globe	A	6.18 (39%)	16.06 ± 0.040	ND
	B		0.053 ± 0	0.013 ± 0
	C		ND	ND
Yellow Globe Hybrid	A	7.54 (53%)	14.16 ± 0.380	0.006 ± 0.020
	B		0.055 ± 0	ND
	C		0.010 ± 0	ND
Sweet Spanish Hybrid	A	4.73 (50%)	9.51 ± 0.090	ND
	B		0.113 ± 0.012	0.008 ± 0.002
	C		0.032 ± 0.001	ND
Red Hamburger	A	2.61 (40%)	6.60 ± 0.180	ND
	B		ND	ND
	C		ND	ND
Walla Walla	A	2.69 (51%)	5.30 ± 0.090	0.067 ± 0.002
	B		0.082 ± 0.008	0.022 ± 0.003
	C		0.001 ± 0	ND
Evergreen Long White Bunching	leaves	0.06 (5%)	1.14 ± 0.007	0.004 ± 0.031
	bulbs	ND	ND	ND

[a] Results are given as mean ± standard deviation for triplicate determinations. A = dry skin; B = outer rings (scales 1–3); C = inner rings (remaining rings); ND = not detectable.

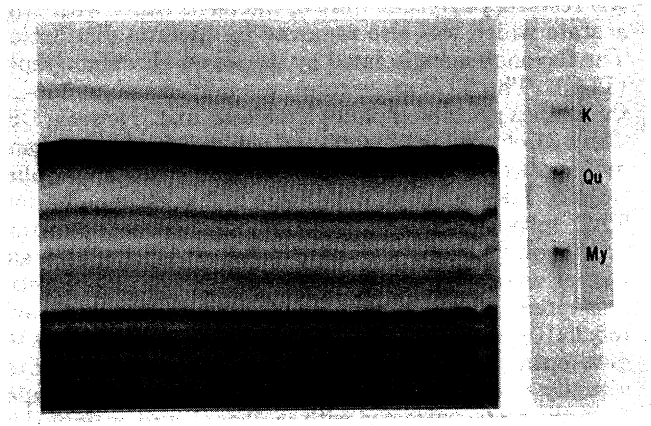

Figure 1. Preparative thin-layer chromatography of hydrolyzed onion skin extract: (K) kaempferol band; (Qu) quercetin band; (My) myricetin (not present).

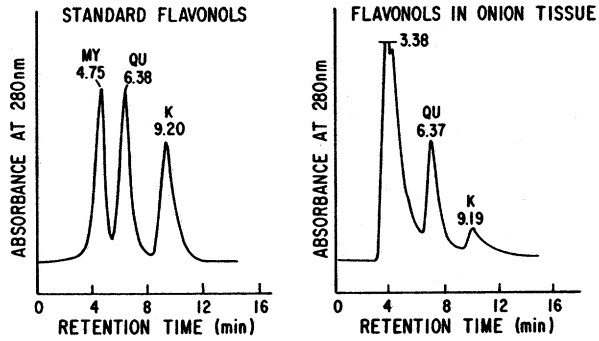

Figure 2. HPLC analysis of hydrolyzed extract of portion B (scales 1–3) of Walla Walla variety: (My) myricetin; (Qu) quercetin; (K) kaempferol.

standards, quercetin, kaempferol, and myricetin (Figure 1). Only two putative flavonols were detected in onion extract: quercetin and kaempferol. The UV spectrum of the fastest moving band, denoted by K, showed absorption maxima at 265 and at 356 nm. This is characteristic of kaempferol (Mabry et al., 1970). The slower moving band (Qu) gave absorption maxima at 254 and 370 nm, indicating the presence of quercetin (Harborne et al., 1975). Some of the other TLC bands also had UV absorption spectra characteristic of flavonols (maxima near 250 and 375 nm), but none of these components gave HPLC retention times similar to that of myricetin. Flavonol separations by HPLC are illustrated in Figure 2. Components tentatively identified as quercetin and kaempferol had retention times of 6.37 and 9.19 min, respectively, the same as those of the standards. The broad band at retention time 3.38 min was isolated and found by separation with TLC and HPLC to consist of compounds having chromatographic properties different from those of myricetin.

Quantitative measurements of the amounts of quercetin and kaempferol present in various portions of eight onion cultivars are summarized in Table I. The skin portions contained large amounts of quercetin although somewhat less than values reported by Herrmann (1958) and by Varshney and Ali (1971). Carmen Hybrid skin had the highest total quercetin content. As much as 53% of the total quercetin in onion skin was present as the aglycon. Carmen Hybrid skin also contained the largest amount of kaempferol. With all cultivars except Red Hamburger, quercetin was detected in portion B (scales 1–3) and C (remaining rings), the former containing more than the latter. A much smaller amount of kaempferol was found in the onion skin and/or first three scales of these samples. The Red Hamburger variety did not contain kaempferol in any portion. The scallion type of onion (Evergreen Long White Bunching) contained quercetin and kaempferol only in the leaves, none being detected in the bulb portion.

The quantity of quercetin and kaempferol in the edible portions of onions is of interest in establishing information on dietary consumption, should these compounds prove to be of toxicological significance. Table II gives the quantities of quercetin and kaempferol in the total edible portions (B and C) of onions. The largest amounts of quercetin were detected in Sweet Spanish Hybrid, Sweet Spanish Utah, and Carmen Hybrid cultivars. Small amounts of kaempferol were found in the edible portions of some onion varieties, with the highest kaempferol content being observed in the Walla Walla variety. These flavonol contents are comparable to the quantities of

Table II. Flavonols in the Total Edible Portion of Onions (mg/kg Fresh Weight)[a]

variety	quercetin	kaempferol
Sweet Spanish Hybrid	62	3
Sweet Spanish Utah	61	ND
Carmen Hybrid	59	ND
Walla Walla	26	7
Yellow Globe Hybrid	25	ND
Early Yellow Globe	15	4
Red Hamburger	ND	ND
Evergreen Long White Bunching		
leaves	2	<1
bulbs	ND	ND

[a] ND = not detectable.

quercetin and kaempferol found in the edible portions of other fruits and vegetables known to be rich in flavonols (Herrmann, 1976), for example, sour cherry (80 and 17), black currant (68 and 10), apricot (53 and 2), apple peel (263 and 7), Brussels sprouts (25 and 40), and pea pods (130 and 5 mg of quercetin and kaempferol, respectively, per kg fresh weight).

Knowledge of varietal differences in the flavonol distribution in onions may be of potential value to breeders should they be required to select for reduced flavonol content. However, differences in flavonol content within a commodity need to be understood in terms of maturation and environmental effects before varietal differences can be exploited.

Registry No. Quercetin, 117-39-5; kaempherol, 520-18-3; myricetin, 529-44-2.

LITERATURE CITED

Harborne, J. B.; Mabry, T. J.; Mabry, H. "The Flavonoids"; Academic Press: New York, 1975.

Hardigree, A. A.; Epler, J. L. *Mutat. Res.* **1978**, *58*, 231.

Herrmann, K. *Arch Pharm. (Weinheim, Ger.)* **1958**, *291*, 238.

Herrmann, K. *J. Food Technol.* **1976**, *11*, 433.

Koeppen, B. H.; Herrmann, K. *Z. Lebensm.-Unters. -Forsch.* **1977**, *164*, 263.

Mabry, T. J.; Markham, K. R.; Thomas, M. B. "The Systematic Identification of Flavonoids"; Springer: Berlin, 1970.

MacGregor, J. T.; Jurd, L. *Mutat. Res.* **1978**, *54*, 297.

Mohr, H. "The Physiology of Plant Growth and Development"; Wilkins, M. B., Ed.; McGraw-Hill: London, 1969.

Siegelman, H. W. "Biochemistry of Phenolic Compounds"; Harborne, J. B., Ed.; Academic Press: London, 1964.

Varshney, I. P.; Ali, T. *Indian J. Appl. Chem.* **1971**, *34*, 142.

Wulf, L. W.; Nagel, C. W. *J. Chromatogr.* **1976**, *116*, 271.

Received for review May 23, 1983. Revised manuscript received October 6, 1983. Accepted October 26, 1983. Presented at the 184th National Meeting of the American Chemical Society, Kansas City, MO, Sept 12–17, 1982. Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.